THE MORPHOLOGY AND LIFE HISTORY OF *NOSEMA APIS* AND THE SIGNIFICANCE OF ITS VARIOUS STAGES IN THE SO-CALLED 'ISLE OF WIGHT' DISEASE IN BEES *(MICROSPORIDIOSIS)*

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PLATES XIV, XV, XVI

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I. INTRODUCTION

The subject of this paper is the small, one-celled, parasitic organism, known as *Nosema apis,* which is responsible for a fatal disease among bees in Great Britain. The parasite was first discovered by the writers in 1906 in diseased bees from the Isle of Wight, where an epizoötic was raging among the apiaries. From then onwards, as opportunity afforded, we have made a study of the parasite, a matter of much difficulty, as the young forms of the parasite are frail, and decompose very rapidly.

Nosema apis is a minute organism belonging to the Protozoa. One stage of the parasite takes the form of very resistant tough, oval bodies termed spores, which serve to infect new hosts. On this account the *Nosema* is put in that section of the Protozoa known as the *Sporozoa.* The spores are very small and have a somewhat complicated structure, a feature which places the parasite in the group known as the *Microsporidia.*

Among the *Microsporidia* are several organisms which are fatal to fishes, while others attack insects. One of the best known *Microsporidia* is a very near ally of *Nosema apis*, namely, *Nosema bombycis*, the organism that caused great mortality among the silkworms and enormous damage to the silk industry of France in the middle of last century. The genus *Nosema*, then, is associated with two fatal maladies of insects, as *Nosema bombycis* causes the pébrine of silkworms, while *Nosema apis* is a destructive agent among bees. In our paper of 1911, we named this disease of bees Microsporidiosis.

In this paper (which is based on our Report to the Board of Agriculture) care has been taken to explain all technical terms, and sometimes the explanation has been repeated. We trust that this procedure will be no offence to the scientific worker, and that it will be of service to those who have a keen interest in the bee folk without any special scientific leanings. Should the zoological terms, even under such conditions, be a worry, we would suggest that readers turn to our papers immediately preceding* and succeeding*, which are certainly in no way of a technical nature, but possess a distinct practical interest.

II. MATERIAL AND METHODS

During the early part of our research we were much hampered by paucity of material. As we could get very few infected bees—owing to the fear of the beekeepers that removal of bees would mean spread of disease—we used much of our first material for cross-infection experiments to decide whether or no *Nosema apis* really was pathogenic (pp. 155-7). The life history of the parasite was worked out to a large extent, with results which subsequent

* This Journal pp. 145-161 and 197-214

work has fully confirmed. It should be remarked that while an abundance of dead bees was sometimes available these were of use only for gross diagnosis, and that live bees were absolutely essential to get finer details and all the various young (but deadly) stages in the life history.

Much time was spent in the examination of fresh material. Bees were chloroformed rapidly and then dissected. Smears of the gut walls were prepared and examined fresh. Serial teased portions of the gut were made into hanging drops on slides, or kept in small paraffin-edged cells sealed under a cover-glass. *Intra vitam* staining with methylene blue was sometimes of service.

Detailed examination of teased and sectioned organs, other than those of the alimentary tract were also made (see Sect. V, p. 180). Eggs, larvae sealed and unsealed, adult workers, drones, and a few queens, have all been examined in detail.

Stained preparations were also made, and the guts of some bees, both normal and infected, sectioned serially. One important point was that detailed examination of normal bees was made, so that errors of interpretation of various structures have been excluded.

For fixatives, osmic acid vapour followed by absolute alcohol, corrosive-acetic-alcohol and Bouin's picro-formol-acetic fluid were employed. The stains that gave the best results were Giemsa, haematoxylin, modified Romanowsky and glycerin haematin.

Sections were usually very disappointing, partly because the infected epithelial cells were often shed into the lumen of the gut. In any case there was difficulty in differentiating young amoeboid forms from the tissues they had recently invaded. Spores were obvious enough wherever they occurred.

For the elucidation of certain features, such as a thread-like coiled structure in the spore known as a polar filament, special methods were used, and are briefly indicated in the part dealing with these structures (p. 174).

III. MORPHOLOGY AND LIFE HISTORY OF *NOSEMA APIS*

Several stages can be recognised in the life-history of *Nosema apis*. The first stage is that of the tiny, amoeboid germ (Pl. XIV, figs. 1-3) that issues from the resistant spore. This amoebula gives rise to uninucleate daughter forms, known as planonts because of

their migratory or wandering habits. Planonts can increase in numbers by division, and correspond roughly to the young, feeding and growing, or early trophozoite phase of other Sporozoa.

The Planonts

Each amoebula as it issues from the spore, shows two nuclei as refractile spots (fig. 3). The planont moves by pseudopodia, one pseudopodium only being formed at one time, as a rule (fig. 3), and this generally at one part only of the organism. The small amoeboid body may behave in one of two ways:—

1. The two nuclei may fuse together—a process often known as autogamy, but strictly one of karyogamy—after which the parasite creeps slowly between the cells of the gut and ultimately penetrates them. Division of the uninucleate amoebula may occur while the parasite is free in the lumen of the gut of the host.

2. Protoplasm may collect around each of the nuclei of the amoebula, and then division occur, so that two daughter forms are produced. Each of these daughter forms may divide by binary or multiple fission, the latter giving rise to a colony of young planonts (fig. 1), each of which moves about over the epithelial surface of the gut, and finally penetrates between the cells or directly enters them (fig. 4) and becomes intracellular. Some of the planonts can also pass between the cells of the gut and reach the body cavity or haemocoel.

The small planonts ultimately become round or oval (figs. 4, 5), and are about 0·75 μ-2·5 μ in diameter. When active, their movements are slow, and the pseudopodia, probably on account of the smallness of the organism, show little differentiation into ectoplasm and endoplasm, as a rule, though ectoplasm may be quite well seen occasionally in the pseudopodia. Small vacuoles are sometimes present. They are not contractile so far as could be observed. The nucleus is small and consists of a small chromatin mass or karyosome (figs. 2-6) suspended in a less dense substance, the nucleoplasm.

Free planonts on the surface of the gut epithelium, or detached, and so smeared with the rest of the gut contents, stain fairly well with Romanowsky stains. When they become intracellular they stain only moderately, and can be distinguished from the cell

contents only with some trouble. The method of penetration of a cell by a planont is most difficult of observation, though it has been seen in life on a few occasions.

A point of considerable importance is that more than one young planont can enter a single cell. In certain cases (fig. 6) there occurs inside a host cell a number of young parasites which are the original planonts and have not been produced by the division of any older growing form. Multiple penetration by planonts has been observed in life. Since each original individual, after a period of rest and growth, is capable of rapid division which is repeated again by the daughter forms produced, the ultimate result of the multiple infection on the cells invaded is to produce crowded masses of spores (Pl. XV, figs. 53-56, Pl. XVI, figs. 57, 69). These break up the intestinal lining into shred-like masses or a spongy pulp (Pl. XV, fig. 44). Rapid increase in the numbers of the parasites within the same host is ensured by colonies of planonts invading individual cells of the gut, as well as by the multiplication of the parasites later.

It may be mentioned that great precautions have been taken to avoid confusion of planonts with other organisms such as yeasts. The planonts can be distinguished from yeasts (1) by their movements, (2) by the stainability of their nucleus, (3) by chemical tests, of which that for fungus cellulose is the chief. Planonts have no fungus cellulose.

The Meronts

When the planonts have penetrated between the epithelial cells one of two courses may be adopted by them:—(i) They may reach the haemocoel or body cavity of the bee and remain there in a resting condition for some time. They lose their motility temporarily, become rounded or oval (Pl. XIV, fig. 2) and lie quiescent. After an interval their activity returns, and from the haemocoel they retreat between the cells to the epithelium of the gut, which they gradually penetrate. (ii) Other planonts are capable of penetrating the gut direct from the lumen or from between the epithelial cells. In either case, the active motile planont becomes passive, loses its pseudopodia and enters on a growing stage (fig. 5), which is

followed by multiplication after a short time. The intracellular parasite is now called a meront or dividing form (figs. 7, 8).

A meront at first is uninucleate (figs. 7-9), and in this condition resembles the planont. The structure of the meront at first only appears to differ from that of the planont in that the nucleus gradually becomes more chromatic and compact in nature. The organism, which has now entered upon its trophic or feeding phase, rapidly increases in size, and then begins to multiply by a process known as merogony (or schizogony). The method of multiplication, wherein meronts by division give rise to daughter meronts, may follow one of three main types:—

I. The simplest form of production of daughter meronts is by *binary fission*, but even here there is considerable variation in the forms produced, thus:—

A rounded meront usually has a round chromatic nucleus (figs. 7, 8). This nucleus becomes bowed and from an approximately central position becomes nearer the periphery. The chromatin concentrates into both ends of the bow, which is thus dumb-bell shaped (fig. 8). An indentation appears opposite the thin strand that connects the two heads of the dumb-bell, the constriction deepens, the chromatin masses separate completely (Pl. XIV, figs. 15-18), the invading ectoplasmic areas meet, and two daughter meronts are thus produced. These rapidly become either oval or rounded like their parent. The nucleus of such meronts may become rounded, or it may form an elongate rod of rather more scattered chromatin granules.

A second and common variation is seen when a somewhat elongate meront (figs. 10, 11) divides (figs. 12-14). The process is essentially the same as that previously described, but two meronts which are elongate are produced. The ectoplasm keeps the two in connection often for a considerable time (figs. 21, 22), but ultimately separation is effected.

Very rarely a meront is encountered in which cleavage of protoplasm has commenced before nuclear fission.

II. *Multiple binary fission* producing chains of meronts is found. While this method of multiplication is common in *Nosema bombycis* of the silkworm, it has been our experience to find it relatively uncommon in the *Nosema* of the bees we have examined,

but we may not have examined many bees at this particular stage of infection. The chain originates by binary fission, as previously described, but without separation of the units. The nuclear division is repeated, giving four or more parasites. Chains of three (fig. 30), five (fig. 43), and other odd numbers in a chain arise by irregularity in the sequence of division of individual daughter meronts. Sometimes oval or rounded meronts still slightly connected are encountered, and in each meront nuclear division is either proceeding, so that dumb-bell shaped masses of chromatin are present, or has been completed, giving two nuclei (fig. 26), which may be round or oval. Protoplasmic cleavage is often delayed in these and similar cases (fig. 25).

Again, it has been our experience to find a chain of several meronts in a fresh preparation, and to see it separate into its constituent individuals in the course of a few minutes. Rapid separation of the daughter meronts seems to be characteristic of *N. apis* as we have encountered it.

Pseudo-chains are sometimes produced by the rolling together and adhesion of originally distinct individuals. Great diversity of form in the nuclei enables one to distinguish a pseudo-chain in stained preparations.

III. Sometimes a meront grows relatively large before nuclear division occurs. Two courses are then open to it:—

In the first case, the large meronts show some tendency to be elongate (figs. 37-41). On the other hand, ovoid forms are encountered (figs. 33-36, 42). A special form of large ovoid meront is shown in figs. 33-35. Here, a large meront produces four nuclei (figs. 32, 35), marked cleavage of part of the protoplasm occurs, and a large amount of it concentrates around the four nuclei, giving thus four daughter meronts (figs. 33, 34) which lie within the remains of the parent form. Similar bodies are more common in *N. bombycis*, as described by Stempell, than we have encountered so far in *N. apis*.

Occasionally, a large multinucleate meront (fig. 38) appears to bud. There is then one daughter meront given off at a time terminally (fig. 39) instead of all the daughter forms being produced almost simultaneously (Pl. XV, figs. 46, 48, 52).

In the second case, other meronts show great delay in the

separation of the protoplasm, accompanied by rapid nuclear multiplication (figs. 46-48). The result is that a large body suggestive of the multinucleate schizont of a Schizogregarine or Coccidium is produced. Around each of the nuclei of the meront of *N. apis* a spore is ultimately formed. In *N. apis* we have found these large multinucleate bodies fairly often, but they are not found in all infected bees and, when present, they usually co-exist with ordinary meronts. These large meronts may be either intercellular or, as we have found more commonly, within cells of the epithelium (fig. 47). As the epithelial cells are shed naturally into the gut, the large meronts can be found free in the lumen of the gut. When the spores are formed, they lie within the cavity occupied by the original meront, and the whole structure finally forms a large, often circular, aggregation of spores (figs. 52-55). Stempell has described similar multinucleate bodies in *Nosema bombycis*, but is inclined to regard them as abnormal forms. At present, we consider the large structures of *N. apis* as merely variants in the method of growth and division, preceding spore formation, which variation seems to us to depend very largely on the factors of the space and nourishment available for the parasite.

In some cases a single cell may become parasitised by several planonts in succession, with the result that recently entered planonts, meronts of all ages, and even mature spores may be co-existing within the same cell (figs. 45, 49). We have found that some of the largest uninucleate meronts (figs. 50, 51) just about to become spores attain a length of 7·5 μ, but there is much variation in size.

It is of interest and practical importance to note that many bees contain only the meront forms of *N. apis* at the time of their death. The tissue destruction due to these young forms of *N. apis* is very extensive, and produces weakness and exhaustion. In this respect the action of the meronts of *N. apis* may be well compared with those of the merozoites of *Eimeria avium* which are responsible for the death of young grouse, fowls, pheasants and pigeons.

Spore Formation

Usually, after merogony has proceeded for some time, *Nosema apis* begins to prepare for life outside its present host by the

production of resting and resistant forms of the parasite known as spores. In other words, merogony is succeeded by a process of sporogony.

Two variations in the method of spore formation are found in *Nosema apis*, corresponding in the main to two of the different forms of merogony.

In the first case, a single oval or rounded uninucleate meront prepares to form a single spore (Pl. XV, figs. 50, 51). At this stage it represents the pansporoblast or sporont of other *Myxosporidia* and *Microsporidia*. The protoplasm is finely granular, the nucleus is distinct and at first single, though ultimately five nuclei are produced. The meront then becomes the sporoblast and the protoplasm contracts to a slight extent. A thin spore-wall or sporocyst is then secreted external to the cytoplasm, and thus from each uninucleate meront a single spore is produced. Figs. 53-56 represent groups of such spores. Great internal nuclear changes occur during the secretion of the sporocyst wall, but these are gradually obscured by the increasing opacity of the spore.

A second variation has been mentioned in that type of merogony wherein the original meront increases in size and undergoes repeated nuclear division, but separation of the protoplasm and nuclei as daughter meronts is prevented or delayed. Ultimately around each nucleus with its protoplasm areas of delimitation are produced (Pl. XV, figs. 46, 48) by the gradual concentration of the protoplasm, and then the secretion by it of spore walls (fig. 52). Such uninucleate masses before the secretion of the sporocysts are called sporoblasts. Nuclear changes are obscured in the living organism to a large extent, as in the previous case, but are of the same type and will be described in some detail later. When the sporocyst is fully formed, the sporoblast becomes the spore.

The internal structure of a mature spore is practically invisible in life and the general impression conveyed by microscopic examination of fresh preparations is that of a mass of spores, resembling rice grains, somewhat irregularly disposed lying within the remains of the host cell that was originally invaded by the single meront (Pl. XV, figs. 52-54, Pl. XVI, figs. 57, 69). When a large, multinucleate meront has been developed within a cavity

between cells, the appearance is that of a mass of spores with a slight haze or halo around them produced by the dead remains of the parent meront.

As before mentioned, multiple infection of single host cells by several planonts giving rise to meronts has been found (Pl. XV, figs. 45, 49). In these cases of multiple infection, the meronts when dividing may interfere mutually with the separation of the daughter meronts, causing temporary fusion of the various individuals. The result is that a fusion mass, or plasmodium, is produced. Each of the nuclei in such a plasmodium induces a concentration of protoplasm around itself, becomes a sporoblast, and finally a spore, as in the previous cases.

It is thus seen that the meront becomes successively the pansporoblast, the sporoblast and the single spore, and thus *Nosema apis* is placed in the group *Monosporogenea*, established by Pérez in 1905. The great power of merogony possessed by *N. apis* compensates for the production of one spore only from the pansporoblast.

Spore Structure

The young spore is a somewhat elongate, usually oval body (Pl. XV, figs. 53-55, Pl. XVI, figs. 57, 69), sometimes more pointed at one end than at the other (Pl. XV, fig. 56, Pl. XVI, figs. 60, 62, 66), from 4 μ to 6 μ long by 2 μ to 4 μ broad. Very occasionally a spore may be 7 μ long.

When young, the contents of the spores are finely granular and a single nucleus can be seen within them in life (fig. 61), which nucleus stains well. The granular cytoplasm of the spore gradually concentrates towards one end which we may term anterior. This end may be somewhat pointed (figs. 61, 62). The other end then becomes filled with liquid and forms a large posterior vacuole, which is refractile in life. Soon tiny vacuoles form in the concentrated mass of protoplasm and these gradually fuse together to form an anterior vacuole which is smaller than the posterior one. This anterior vacuole is the polar capsule (figs. 58, 62, 63), and is so called because a long, spirally coiled thread termed the polar filament is ejected through it. In life the polar capsule seems far more difficult to detect than the larger posterior vacuole. The formation of the vacuoles completed, the cytoplasm

appears as a ring-shaped or girdle-like mass (figs. 63, 71, 77), with a vacuole at either end. At about this stage nuclear multiplication commences, and at the same time the chitinoid sporocyst is secreted much more rapidly than hitherto. The nucelus first begins to elongate and becomes bowed. Gradually the chromatin concentrates into the ends, and the nucleus becomes dumb-bell like, and ultimately the ends separate. This division, then, results in a binucleate spore (figs. 63, 64). From one of the two nuclei a bud next arises. This separates from the parent nucleus and passes towards the anterior vacuole. The nucleus thus budded off is the polar capsule or capsulogenous nucleus. The second nucleus from the original division divides into two small nuclei (figs. 65-68), which at first are rounded and are embedded in the cytoplasm, or, as it is better called, the sporoplasm. These small nuclei gradually pass to the periphery, elongating and becoming thread-like as they do so (figs. 68, 72, 73). As they control the subsequent growth of the sporocyst, they may be termed the sporocyst nuclei, and are apparently comparable with the valvular or parietal cell nuclei of the Myxosporidia (pp. 176-8). The remaining nucleus of the sporoplasm also divides into two, so that the sporoplasm is binucleate and thus the full-grown spore contains five nuclei (figs. 65-67, 77). These nuclei, as will be gathered, are not always quite the same size and shape, and do not all persist for the same length of time, so that five nuclei are not always seen in the spore together. Some apparently ripe spores show only four nuclei (Pl. XVI, figs. 71-73, 74, 85). The polar capsule nucleus and the sporocyst nuclei after fulfilling their function may degenerate gradually.

By the time that the division of the nuclei is complete, the sporocyst has thickened enormously and the sporoplasm, which secretes the wall, tends to shrink away from the wall in some cases Pl. XVI, figs. 72, 74, 85). The increasing density of the wall renders the details of the nuclear structures within very difficult of observation, and prolonged staining with or without a preliminary treatment with potash or creosote is necessary. The fully formed fresh spore is highly refractile, and a number of them seen with the microscope resembles a collection of polished grains of rice. Each spore is about one-thousandth the size of a rice-grain.

The quintinucleate character of the spore of *Nosema* is shown diagrammatically in the text figure on p. 177.

The Polar Capsule and Polar Filament

Proceeding from the sporoplasm there is an elongate thread which is spirally coiled and lies usually in the vacuole at the posterior end of the spore. Unfortunately, in life, it is usually invisible within the spore, and even in stained preparations can only be seen with very great difficulty. On this account, Gurley called the *Microsporidia* the *Cryptocystes*. However, the polar filament can be made to extrude artificially by the aid of reagents, of which we have found iodine water and very dilute acetic acid the most useful. The polar filament extends from the extreme anterior end of the spore towards the posterior end. From the edge of the sporocyst it passes as a straight rod (fig. 73) backwards through the polar capsule, and after reaching beyond the greater mass of the sporoplasm it becomes spirally coiled on itself in the vacuole (figs. 72, 73). It measures about 60 μ in some of those that we have examined. Polar filaments are very difficult of observation under any circumstances. Figs. 75 and 76 represent the same spore before and after treatment with iodine water.

While the spore is at rest, the polar filament remains quiescent. Occasionally, as at times of rapid induced currents in the bee's gut, the spores thrust out their polar filaments (figs. 79-87), which hook into the cement between the epithelial cells and temporarily anchor the parasite. The gradual protrusion of the polar filament (figs. 79-80) can be seen at times in fresh preparations, and is more easily observed after the addition of iodine water or weak acetic acid.

Again, the polar filament is extruded when the amoebula is about to leave the spore. After being vigorously flung out, the attached end of the polar filament either snaps off or is forced out of the spore by the movements of the sporoplasm within, and thus a spore is seen with a separated polar filament in its vicinity (fig. 89). A spore whose polar filament has escaped (fig. 90) may show a very small pore marking the point of exit. The amoebula leaves the spore after the polar filament has been extruded, and moves actively in an amoeboid manner away from the spore. Spores devoid of amoebulae can be found, and then the sporocysts, sometimes with their two sporocyst nuclei, are recognised as empty by their decreased refractivity and by the minute pores whence the polar filaments escaped.

Variation in the Appearance of Spores

While a very large proportion of spores show the features as detailed previously, yet there are certain well marked differences, in the form of young spores, more particularly, which are the results partly of the conditions of nutriment under which the parasites develop, and of the space available for their development.

(1) Young spores often are not perfectly oval, but are more pointed at one end, or somewhat egg-shaped (fig. 72). Others, on the contrary, become much more rounded (fig. 79). While the egg-shaped condition is fairly common, the rounded form is rarer.

(2) Young spores may not show the vacuole and polar capsule at once, but protoplasmic strands may cross either cavity, and so the appearance of several vacuoles is produced (fig. 70). Ultimately these vacuoles fuse, and the final result is the production of a single large posterior vacuole and a polar capsule.

(3) While a small but not very obvious thickening (figs. 79-81) was present at the attached end of every polar filament, in a very few cases this thickening appeared enlarged (fig. 85), but a much more common condition was to find the thickening very slight, in fact, on occasions it appeared to be absent altogether.

(4) Variations in the position of the sporoplasm are not very great as a rule. Some cases have been observed in which the sporoplasm receded far into the spore, the polar capsule being greatly enlarged. A small posterior vacuole is sometimes present (figs. 59, 63, 67), and the vacuole may be somewhat lateral (fig. 68). Spores in which the polar capsule was slight (figs. 58, 64) have also been found.

(5) There is much variation in the position of the nuclei contained in the spore. The most typical case of the quintinucleate spore is where the sporocyst nuclei are laterally placed, the capsulogenous nucleus is near the polar capsule and the sporoplasmic nuclei more or less central (figs. 67, 74, 77, 89). But the polar capsule nucleus not infrequently lies in the sporoplasm, and is only differentiated from the sporoplasmic nuclei by its somewhat smaller size and gradual disappearance. Similarly the sporocyst nuclei may be rounded (fig. 70) instead of elongate, and they may lie in almost any position in the spore instead of being lateral (figs. 70, 78, 90).

(6) Young spores may show wrinkles in their outer surface in stained preparations. In fresh specimens, such wrinkles are not visible, and they are to be regarded as artifacts due to the action of the fixatives.

We may note that the nuclei of the meronts and spores of *Nosema apis* seem to consist of chromatin, and that most variable (but 'fashionable' at the moment) substance, volutin, is absent.

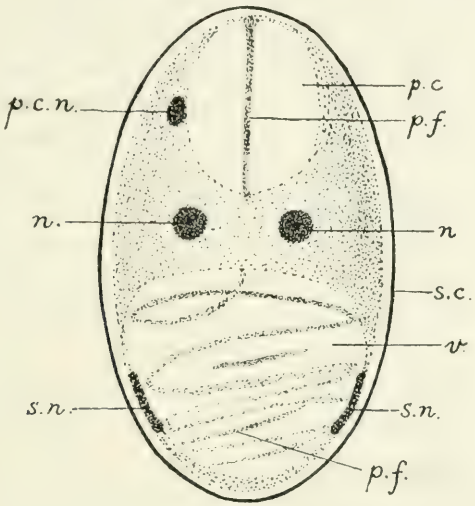
IV. THE HOMOLOGY OF THE MICROSPORIDIAN SPORE AND ITS CONTENTS

The multinucleate condition of the sporont and spore of the *Microsporidia* is, at first sight, perplexing, and though many explanations seem to have been attempted, yet few are satisfactory, and they fail to reveal the undoubted affinity which exists between the *Microsporidia* and the *Myxosporidia*. The Microsporidia, e.g., *Nosema*, on account of their marked parasitism, have developed great reproductive power. In the case of *N. apis* this is seen in the great and varied power of merogony in the host, which compensates for the relatively limited capacity for sporogony, as seen in the production of only one spore (monosporogony) from a sporont or pansporoblast.

Many of the spores of the *Myxosporidia* contain two polar capsules, and two spores are usually formed in each pansporoblast. About ten nuclei are commonly found in such a pansporoblast. We could attempt directly the homology of the five nuclei of the Microsporidian pansporoblast with those of the Myxosporidian pansporoblast, but the comparison is, in our opinion, far more certain and convincing if we consider the annectant form, *Coccomyxa*, whose sporogony has been described by Léger and Hesse (1907).

Coccomyxa morovi, a parasite of the gall-bladder of the Sardine, is a monosporous Myxosporidian, showing many points of resemblance with the Microsporidia. The single, bivalved spore of *Coccomyxa* produced by each pansporoblast contains a clearly visible, single polar capsule. Within the young spore there is a binucleate sporoplasm, two nucleated parietal or valvular cells and one nucleated polar capsule cell. Thus we see that within a developing pansporoblast of *Coccomyxa* there are found five nuclei.

This fact is of the utmost interest and importance, for it reveals in the clearest possible manner, and in a way which apparently has not been clearly pointed out before, the affinity with the microsporidan spore, e.g., the spore of *Nosema apis* (cf. Text-fig.). Both spores contain a binucleate sporoplasm. The single oval sporocyst of *N. apis*, is, we believe, homologous with the bivalve 'shell' or spore coat of *Coccomyxa* and other *Myxosporidia*. In the formation of the spore of *N. apis*, we clearly see two sporocyst



TEXT-FIG. SPORE OF *NOSEMA APIS*

Slightly diagrammatic. As seen after long staining with Romanowsky solution.

p. c. polar capsule or anterior vacuole
v. posterior vacuole
n. sporoplasmic nuclei
s. n. sporocyst (valvular or parietal) nuclei
p. c. n. polar capsule (capsulogenous) nucleus
p. f. polar filament
s. c. sporocyst

nuclei which arrange themselves opposite one another at the periphery of the spore. The sporocyst of *N. apis* is not bivalved, and there is no sutural line, but it is of interest to note that long ago—before we thought of comparing the spores of *Nosema* and *Coccomyxa*—we noticed in a very few cases a more or less longitudinal 'line of weakness' (Pl. XVI, fig. 88) suggestive of the sutural line of the Myxosporidian spore. Lastly, there is a polar

capsule in the spores of *Coccomyxa* and *Nosema*, though in the latter the polar filament is not visible in life, and so is termed cryptocystic. The polar capsule in each case arises from a potential nucleate cell. These homologies throw an interesting light on the phylogeny of the *Microsporidia*.

Before concluding this section we should like to refer briefly to a recent paper by Crawley (1911) on *Sarcocystis rileyi*, parasitic in ducks. In the spore of *Sarcocystis rileyi*, Crawley found two vacuoles and two chromatin masses successively alternating with each other. The first (anterior) vacuole is probably homologous with the polar capsule of the *Cnidosporidia*, the first nucleus is compact and elongate and may be homologous with the compact parietal or sporocyst nuclei of the Microsporidian spore. The second vacuole may be homologised with the posterior vacuole of various Microsporidian spores. The second nucleus, which is vesicular, is probably homologous with the vesicular nuclei of the developing spores of *Myxobolus* or of *Paramyxa* and represents the nuclei of the sporoplasm, which may be germinal in character. These homologies have been discussed by us recently at somewhat greater length in another paper.* They are not without interest in considering the systematic position of the *Sarcosporidia*.

V. DISTRIBUTION OF PARASITE IN THE HOST.

Nosema apis is, at the present time, a specialised parasite, in that its parasitism is restricted almost entirely to the organs of the alimentary tract. In the oesophageal portions of the food canal spores of *Nosema* are occasionally found. These have been freshly ingested. The honey stomach of the bee also rarely contains stages other than the freshly absorbed, resistant spores, though on a few occasions we have found the small amoeboid planonts creeping over the lining of the crop and beginning to penetrate its cells. Beyond the crop is the small lock that leads into the more muscular mid-gut or chyle stomach. The walls of this organ are more easily attacked than those of the fore-gut, and the small amoebulae creep from the spores which are now softened by the digestive juice of the bee, and, after moving about for some time, penetrate the walls, round off, and become meronts. The amoebulae are found in all parts

* Proc. Camb. Philosoph. Soc., (1912), Vol. XVI, pp. 581-584

of the chyle stomach, but it is not uncommon to find one part of the organ swarming with parasites while a very short distance away the tissue is absolutely uninfected. Again, multiple infection of cells in one special area may be common, while a neighbouring portion contains very few, widely distributed parasites.

The chyle stomach narrows to form the small intestine, in which the same condition of distribution prevails. But here, some of the spores which are produced from parasites in the chyle stomach come to rest. Polar filaments are ejected and the spores anchored temporarily. The small, amoeboid germs emerge and proceed either with or without dividing to penetrate new cells of the intestine. This auto-infection of the bee with spores from its already contained parasites only goes on to a limited extent, but it is certainly present.

The hind-gut is rarely a seat of actual invasion by the *Nosema*, but its contents may be milk-white instead of yellowish on account of the *Nosema* spores which have been shed into it.

The chyle stomach is actively secretory in a normal bee. Recent work on the formation of the bees' digestive fluid seems to indicate that the large, secretory cells form the fluid within them in small drops. The drops are not expelled as such, but the cell in which they are formed is detached and falls into the lumen of the gut, where sooner or later its contained fluids are set free by its disintegration. These large, rounded secretory cells become invaded by *N. apis*, and consequently, free, floating in the gut contents, are found globular cells containing young stages of *Nosema*, multiplicative forms of various types and even fully formed spores. It may be mentioned that the bee is constantly renewing and replacing the secretory cells, and it is possible that an active shedding of infected cells may act as a clearance and cause the bee to become healthy for a short time, and so aid in explaining apparent recoveries from attacks of *Nosema apis*. Incidentally the young stages of the parasite are thus lost, and so are seldom seen in sections of the infected gut.

The colon and rectal regions of the gut are practically unattacked by the parasite.

Detailed examination has been made of organs other than those of the main digestive tract. The Malpighian tubules are numerous,

and as they pass off from the gut and have an excretory function, much attention was given to examining them. On one occasion only was any parasite found in the Malpighian tubules, and then meronts and a few spores were found in one tubule only of one bee.

Examination of the salivary glands and wax glands has, so far, proved negative, no form of parasite having been found therein.

The generative organs have been subjected to detailed examination whenever possible. The testes of drones, however, have so far proved negative, although the alimentary tracts of drones are frequently infected. Again, hereditary infection such as occurs in silkworms might be suspected. So far, the material available for examination prevents the expression of a definite opinion. Out of six queens examined by us, in one case only were there indications of *Nosema* in the ovaries, although three of the queens contained spores in the gut. This part of the investigation is of great interest, but much more material is needed before an authoritative opinion can be given. However, it must be noted that while ovarian infection in the queens is questionable at present, there is no doubt that queens can be heavily parasitised by *N. apis* in their alimentary canals, and queens can and do die of the same, though they may be the last in the hive to die. Owing to the infection of the digestive tracts of the queen, there is danger of the eggs becoming soiled with *Nosema* spores just after being laid.

Careful investigation has been made of the haemocoelic fluid of bees. In the first instance much work was done on the haemocoelic fluid of normal, healthy bees, so that the corpuscles of the fluid were well known. The fluid was obtained by snipping off legs from the bees and taking up the drops of fluid that exuded in a fine pipette or directly on to a slide. This fluid was either examined fresh, or after fixation and staining. A similar procedure was adopted with bees known to be unhealthy and suspected of containing *Nosema*—a suspicion that subsequent dissection fully confirmed. The result of examination of fresh preparations was that small, amoeboid bodies, certainly foreign to healthy bees, were found to be present. Examination of stained preparations showed that these bodies were identical in form and structure with the wandering planonts of *Nosema apis*. In

about six instances in which these small bodies were found in the haemocoelic fluid, the chyle stomach of the bees contained a far greater number of planonts, whose movements and appearance were carefully compared with the intruders. We were forced to conclude as a result of this that the planonts could and did pass through the intestinal walls, reach the haemocoelic fluid and remain there. Also, we have found the older stages, the meronts, and in one case one or two spores were found in the haemocoelic fluid. We know well that such development happens in the pébrine of the silkworm, and there seems little reason to suppose that *N. apis* may be more limited in its possible powers of migration than *N. bombycis*.

But *N. bombycis* is much more deadly than *N. apis*, as it already possesses the power of invading every tissue of the body and of destroying even the muscles of the body by its action. Should the power of diffuse infiltration possessed by *N. apis* increase with time, there is the probability of the virulence of the parasite increasing enormously.

Immature bee grubs of varying ages have been examined and found to contain *N. apis*, particularly in the meront stage, while occasionally a few spores were found in the cells of the mid-gut of the young hosts. Whether the larvae were infected by means of their food or hereditarily, there was not enough evidence to show. It seems that the contaminative method is more probable. It may be mentioned that grubs from one particular infected hive appeared somewhat smaller than normal bee larvae.

Muscular tissues of diseased bees also have been examined repeatedly. Except in a few instances where the parasites had developed near the muscles or against the sarcolemma of the muscles of the gut, the muscular system appeared free from the parasites. *N. apis* in this respect is very unlike *N. bombycis*, by whose action the muscle substance is destroyed and ultimately the sarcolemma becomes largely filled with masses of meronts instead of muscle substance.

To sum up, the main alimentary tract, particularly the chyle stomach and intestine, are the chief parts of the bee infected by the parasite. The gut diverticula appear to be free from parasites. But as the parasites may be found in the haemocoelic fluid, there is the

possibility of the extension of the *Nosema* into hitherto uninfected organs.

V. INTER-RELATION OF THE HOST AND THE PARASITE

Action and reaction are equal and opposite according to physical laws. We may then enquire as to what relationship or interaction exists between *N. apis* and its host. In examining fresh preparations of bee guts infected with *Nosema apis* we have often found the large intestinal cells containing one meront lying free in the gut lumen. Each meront appeared to be lying in a clear space and had the appearance of being surrounded by a halo. No space was seen around the meront which had only just become intracellular. The more mature the meront, the more marked was the space.

The production of the halo around the meront may be due to two causes:—

(1) Stempell, when working on *N. bombycis*, notes a similar space and ascribes it to the action of a ferment produced by the parasite. The same may be true in the case of *N. apis*. The said ferment decomposes the protoplasm, and the dissolved products serve as nourishment for the parasite. Support is given to this hypothesis by the gradual appearance of the space around the organism, suggesting, as it does, the progressive destruction of the surrounding tissue.

(2) The clear area may be due to the removal by simple absorption from the cytoplasm of the invaded cell of various granular constituents, used by the parasite as food. The organism would then exercise a sort of selective absorptive power, which would alter considerably the concentration of the liquids external to itself.

This view is supported by the fact that when the cells containing the parasites burst, and so discharge the parasites into the lumen of the gut, part of the 'halo' passes out as an investing sheath around the parasite, while a small portion remains behind. The alteration of the fluidity of the nearest cytoplasmic zone of the host-cell would allow of this occurence. The 'halo' is not an integral part of the parasite, for it gradually disappears in water or normal salt solution, and the process can be hastened by warming.

The meront is then left with a clear-cut ectoplasmic outline which does not alter.

It might be suggested that the clear area or 'halo' is an attempt on the part of the host to shut off the parasite. We do not think such is the case, for the parasite certainly grows much more rapidly during the period when the halo is large than when it is smaller. This would hardly be the case were its nourishment being restricted in quantity or quality.

The tissues of the host undoubtedly offer some resistance to the parasite and the result is the production of intercellular multinucleate meronts and spores, as mentioned previously. But the parasite is, even in this condition, able to mature spores, and spore formation may be interpreted as due to the reaction of the host upon the parasite, causing the latter to shorten its active career in that particular host and to assume a form suitable for conveyance to a fresh bee.

The passage of the spores outwards into the lumen of the gut causes tears and gaps to appear in the intestinal wall. The bee, however, has great powers of re-forming cells, owing to the method by which its digestive juice is formed, and consequently the number of torn cells as seen in sections is not necessarily great. Injured cells are merely voided and new ones formed to replace them. When an intense infection is present, the bee seems to lose its power of re-forming cells, and, as a result, the digestive processes are far more deranged, the bee weakens rapidly and dies. The chyle stomach at this time may be full of *Nosema* spores when its normal reddish colour disappears, and it becomes chalk-white in colour.

The presence of the parasite in the haemocoelic fluid has an important bearing on its possible pathogenic effects. *Nosema apis*, at present, appears to be largely confined to the digestive tract, but, as it is able to live in the haemocoelic fluid, there are great possibilities of it becoming like *N. bombycis*, which is capable of attacking any and every tissue of the silkworm, and, therefore, is much more deadly. The deadliness of a parasite depends in part on its power of multiplication within one host. This power is very great in *N. apis*, and multiplication is brought about in many ways. The second factor influencing the fatal or non-fatal effect of a parasite is the extent of its distribution. When the

parasite can penetrate every tissue so that it is diffusely infiltrated throughout the host, the damaging capacity is enormously greater than when the parasite is local. As *Nosema apis* varies in its power of diffuse infiltration (as shown by its presence in the haemocoelic fluid, and, on one occasion, in the Malpighian tubules), its potentialities as a deadly organism may increase.

Much time and thought have been given to the consideration of the variation in virulence of *N. apis* in bees, but little definite evidence can be produced in explanation. However, it is known that in certain other protozoal diseases, such as the various forms of Trypanosomiasis, the newer the parasite is to the host, the more deadly is it found to be. Also, it has been shown that, after a time, the host acquires the power of tolerating the parasite to a greater or less extent. Arguing by analogy with *N. bombycis*, which is fatal to silkworms in practically all European cases, it would seem probable that *Nosema apis* has been a parasite of bees for a longer period than *N. bombycis* has of silkworms. If such be the case, there is, then, the possibility of an immune race of bees developing, and, indeed, certain experiments in cross-infection have shown that some bees may feed on honey containing thousands of *Nosema* spores and yet be unaffected by them. Such bees may be considered immune. Probably many Australian bees are immune to the effects of *N. apis*. Also, if a weak strain of bees is infected with *Nosema*, the disease could and would run riot among them, for any deadly parasite can easily overcome the resistance of a weak host. Immune bees are very dangerous, as they act as parasite-carriers.

Bees, in the early spring, are weaker after their hibernation than they are in the summer. Consequently, if they have harboured *Nosema* in their mid-guts during winter, or acquire it very soon after their awakening, the parasite may be expected to get the upper hand of the bees, and an outbreak of disease is reported. Bad seasons have a similar effect in lowering the vitality of the bees and so rendering them easier victims to microsporidiosis.

VII. SUMMARY OF THE LIFE CYCLE AND ITS SIGNIFICANCE

The most conspicuous stage in the life history of *Nosema apis* is the spore. This may form the starting point of the life cycle. When the spore is taken up mixed with honey or pollen from flowers or from comb in the hive, or sucked in by a bee engaged in cleansing another soiled or infected bee, the spore passes forwards into the chyle stomach of the bee before much change occurs in it, as a rule.

Within the chyle stomach, the spore wall is softened by the action of the digestive juice of the bee, which penetrates within to the contents. Stimulated by the juice, the sporoplasm apparently presses on the vacuole, with the result that the polar filament is forcibly ejected. It serves for a short time as an organ of attachment, fixing the spore to the gut wall. The sporoplasm concentrates and moves forwards, whereby the polar filament is forced still further outwards and becomes disconnected from the spore. The sporoplasm, retaining two of the nuclei, creeps out from the sporocyst, leaving the two sporocyst nuclei behind. The free sporoplasm becomes amoeboid, and the binucleate amoebula creeps about over the intestinal surface. The two nuclei may fuse, or more often, the amoebula proceeds to form more daughter amoebulae without previous fusion of nuclei. The final active amoebulae are small, roundish organisms, each with a single nucleus containing a karyosome. Each amoebula is capable of amoeboid movement. It penetrates the cells of the gut or passes between them, and, finally, either enters one of the cells or goes beyond and floats in the haemocoelic fluid.

Assuming that it enters an epithelial cell of the gut, it becomes rounded and passive therein, and, after a period of growth, during which time it is known as the trophozoite, it commences to multiply by several methods, resulting, usually, in a collection of several daughter individuals or meronts, or in chains of meronts. The chain condition is less common than the separate forms, in our experience, so far. Each meront is uninucleate. Alternatively, a meront may form a large multinucleate body, in which cleavage into daughter meronts does not occur at once. Such bodies may be intercellular or intracellular.

After a period of active growth and division, producing uninucleate meronts, spore formation begins. The organism is now called a pansporoblast. Active, amitotic,* nuclear division occurs, the result of which is ultimately to produce five nuclei. Two vacuoles form also in the young developing spore, a large one at one end, called the posterior vacuole, and a small one at the opposite end, termed the polar capsule. The living body substance or sporoplasm then forms a somewhat ring-shaped mass between the two vacuoles, and in it the five nuclei arising from the division of the original one are lodged. Two nuclei migrate to the sides. These become elongated, and may be termed the sporocyst nuclei. Of the other three nuclei, one controls for a time the polar capsule, and the other two the sporoplasm. The polar capsule gradually forms the spiral polar filament, which extends down into the vacuole. While these nuclear changes are taking place in the sporoblast or young spore, the latter is forming a coat around itself. This coat or sporocyst gradually thickens and obscures the nuclei beneath, and the final result is that, from one pansporoblast, a single spore, resembling a rice grain in shape, with a shining refractile envelope or sporocyst, is produced. In this condition the spore passes into the lumen of the gut, is voided with the faeces, and remains a source of infection for some time.

It will thus be realised that there are two distinct phases in the life of *Nosema apis* within the bee. This feature *Nosema* holds in common with other protozoal parasites, such as the Coccidium fatal to game-birds and poultry.

The first part of the life of the *Nosema* is occupied with growth and active division, so that the number of parasites within the host is enormously increased. This multiplicative stage, termed merogony, is the one that is most dangerous to the host. The young stages of the parasite, alone, are sufficient to kill the bee in many cases, and the parasite, as a result, many never reach the final stage of its development, the spore. Young grouse, similarly, are killed by the multiplying stages of *Eimeria* (*Coccidium*) *avium*, and cattle by similar forms of another *Eimeria*.

* The process of nuclear division may really be a sort of primitive mitosis, intermediate in character between mitosis and amitosis. The organism is too small to show further details of nuclear division.

When the power of the parasite to multiply further in the one host is exhausted, or when the bee can no longer supply it with food, it becomes necessary for the *Nosema* to leave its host and to renew its development in a fresh bee. Consequently, the parasite must protect itself in order to survive the period between leaving one host and entering the next. As a result of this need, it forms a hard, outer covering or spore coat, and becomes a spore. The spores are highly resistant to outside conditions, can live for some time without losing their infective power, and so can become new sources of infection for other bees.

In other words, the merogony of *N. apis* serves for the infection of, and has fatal effects on, a singe host; sporogony is a means for the transference of the parasite to new hosts.

VIII. THE METHODS OF INFECTION

The method by which the *Nosema apis* enters the bee appears to be purely contaminative, that is, the parasites gain access to the host either with the food or drink of the bee, or by the bees licking one another or removing drops of infective excrement from their own bodies, or absorbing spores during cleansing operations within the hive.

As the methods of spreading *Nosema* spores are of great importance in combating disease, we have devoted a short paper to the subject (pp. 197-214), and merely give here a very brief outline of our results.

Bees, when healthy, rarely soil the combs or the interior of their hives. But, when *Nosema* is present, excrement is voided anywhere, and alighting boards, frames, combs and their contents, alike, may be bespattered, and hence infective. When food stores are thus contaminated, infection may continue over a long period.

Healthy bees frequently clean themselves and try to cleanse their neighbours, the tongue being largely used in this process. *Nosema* spores can thus be carried into the alimentary tracts of new hosts.

Infected drinking places are centres of infection for other bees, especially if the water be somewhat exposed and warm. Cold water is disliked, and warmer water, though less clean, is taken

in preference. Heavily infected drinking places have been examined by us.

Robber bees not only further deprive weak colonies of their food, but also carry *Nosema* spores into the new hive as well as the stolen honey. Weak or 'dead' hives are a source of danger to other communities so long as any stores remain in them, or as long as they can afford a refuge either to other bees or to other insects, such as ants.

Plants around infected hives may become heavily contaminated with faeces from the parasitised forager bees. Lilac, gooseberry, and apple blossoms, among others, have been found by us with the anthers encrusted with bee faeces containing spores of *N. apis*, and thus proving a potent source of disease to healthy bees visiting the blossoms.

Nosema spores are extraordinarily light, and are easily distributed by the wind. This method of distribution we have investigated on several occasions, and by detailed examination of the district around certain hives have traced the spores between one hive and another. Wind-borne spores, also, may be deposited in drinking places, where they are in a position for ready absorption by the bees.

Infected drones, by dropping excrement containing spores within their own hives and by visiting several hives in succession, as they commonly do, can also serve as agents in disseminating the spores of *Nosema apis*.

Ants and wax moths, both of which visit hives, can swallow *Nosema* spores but are unharmed by them. The spores pass unchanged through the alimentary tracts of these insects, and are ejected with their faeces in other places.

Human agency has added to the spread of the disease also. The use of old (and sometimes infected) comb and the recolonising of 'dead' hives without previous vigorous cleansing, preferably by fire, has sacrificed many new stocks of bees. The moving of bees to different parts of the country for the purpose of procuring more honey or honey of a particular kind, e.g. heather honey, has introduced parasite carriers into new areas and caused outbreaks. The sale of bees from infected districts also had added its quota to the spread of disease, and the best intentions underlying the sale—

such as the accounts that 'the bees did not like the pollen of this neighbourhood and were getting weak, so we sold them and sent them to X, where there is better pollen'—merely aided in the spreading of disease to new areas without saving the original sickening bees.

Moving of bees from one infected area to another is not open to such objections as bringing new healthy stock into an infected area. Bees that have survived attacks have acquired, to some extent, partial immunity, and so are more likely to succeed than are stocks in which the bee has not attained some degree of toleration for the parasite.

IX. CONCLUDING REMARKS

In conclusion, we may state that the complete life-cycle of *Nosema apis* in the adult hive bee, *Apis mellifica*, was set forth by us for the first time, and, from all we have seen, the cycle in the larval bee takes the same course as in the adults. Further, mason bees and wasps can become infected with *Nosema apis*. The parasite undergoes exactly the same development in their bodies as it does in the hive bee, a point of some interest, showing, as it does, the great powers of resistance to external influences possessed by the parasite, as well as its ability to live under the different conditions that prevail in the alimentary tracts of hive bees, mason bees and wasps. The problem of the existence of hereditary infection is now one that is claiming our serious attention, and the results of the past seem to be in process of confirmation, but much more material is necessary before this most difficult piece of work can be brought to a successful issue. This, we trust to achieve in the future.

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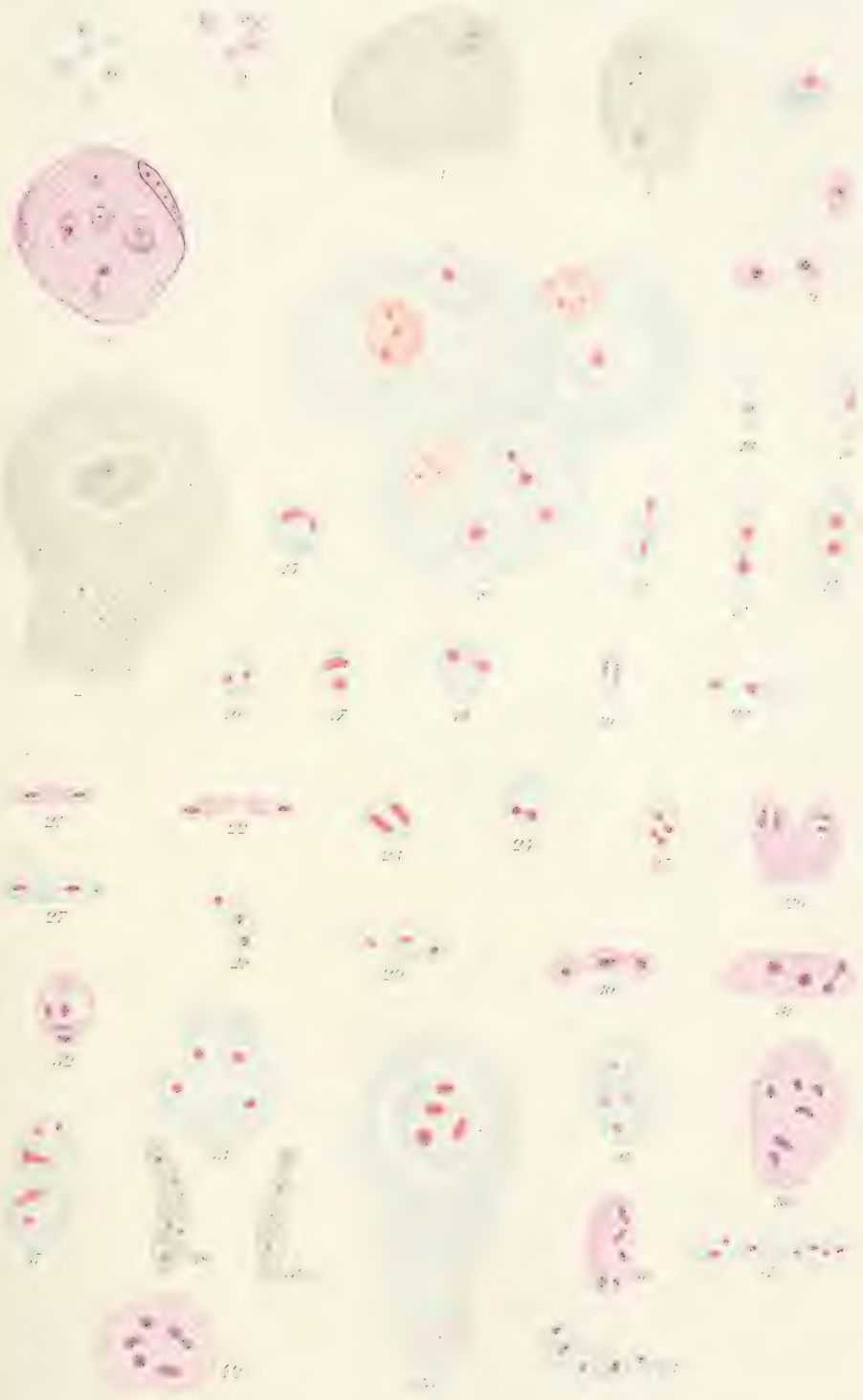
EXPLANATION OF PLATES XIV-XVI

All figures were outlined with the Abbé-Zeiss camera lucida, and 2 mm. apochromatic and $\frac{1}{12}$" achromatic objectives (Zeiss) with compensating oculars 8 and 12 were used.

PLATE XIV

Magnification of figures approximately 1,500 diameters, except Fig. 6, which is 2,150 diameters. Romanowsky stain in most cases.

Fig. 1. Group of planonts of *Nosema apis* from the chyle stomach of *Apis mellifica*.
Fig. 2. Group of planonts from the haemocoel of the bee.
Fig. 3. Binucleate amoebula, showing pseudopodium. Fresh preparation.
Fig. 4. Amoebula becoming round in an epithelial cell.
Fig. 5. Meront with large nucleus.
Fig. 6. Epithelial cell from the chyle stomach of the bee containing planonts and meronts. Haematoxylin.
Fig. 7. Meront lying in a space in an epithelial cell.
Fig. 8. Group of three epithelial cells containing growing meronts. One cell contains three meronts, and of these, one has its nucleus dividing. Nuclei of host cells are faint.
Fig. 9. Three young growing meronts. Haematoxylin.
Figs. 10, 11. Narrow meronts.
Fig. 12. Meront in early stage of division. Nucleus bowed.
Fig. 13. Dumb-bell stage in division of nucleus of meront.
Fig. 14. Broader meront, showing complete separation of daughter nuclei.
Figs. 15-18. Dividing, ovoid meronts.
Fig. 19. Narrow, oval meront with elongate nuclei.
Figs. 20, 21. Narrow meronts.
Fig. 22. Two daughter meronts still united by their ectoplasm.
Fig. 23. Meront with large nuclei.
Fig. 24. Meront with nuclei dividing into two.
Fig. 25. Meront in process of multiple nuclear division.
Fig. 26. Two meronts from one division remaining attached, and each dividing again.
Figs. 27-29. Meronts with three nuclei.
Fig. 30. Chain of three meronts. Haematoxylin.
Fig. 31. Meront with nucleus dividing to form four daughter nuclei. Haematoxylin.
Fig. 32. Quadrinucleate meront showing four vacuoles. This is not common.
Figs. 33, 34. Four daughter meronts enclosed within the remains of their parents.
Fig. 35. Large epithelial cell with quadrinucleate meront.
Fig. 36. Meront with six nuclei. Haematoxylin.
Fig. 37. Somewhat irregular meront from between epithelial cells, destined ultimately to produce five spores.
Fig. 38. Long meront with six nuclei.
Fig. 39. Long meront from which a daughter meront is budding terminally.
Figs. 40-42. Multinucleate meronts of various forms.
Fig. 43. A chain of five daughter meronts which have not yet separated from one another.



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NOSEMA APIS

Plate XV

Magnification approximately 1,500 diameters throughout.

Fig. 44. Piece of tissue from the mid-gut of a bee infected with *Nosema apis*, showing two colonies of meronts lying in spaces and four isolated meronts that have recently penetrated the tissue.

Fig. 45. Single epithelial cell, as shed into the lumen of the gut, containing three dividing meronts, one uninucleate meront, and a practically ripe spore.

Fig. 46. Large meront with daughter meronts differentiating within it.

Fig. 47. Tissue, showing large, multinucleate meronts, in various stages of division.

Fig. 48. A large meront similar to those shown in Fig. 47, but set free into the lumen of the gut.

Fig. 49. Epithelial cell, showing the successive penetration of three planonts producing meronts.

Figs. 50, 51. Meronts about to become spores.

Fig. 52. Large meront with ten daughter meronts within it.

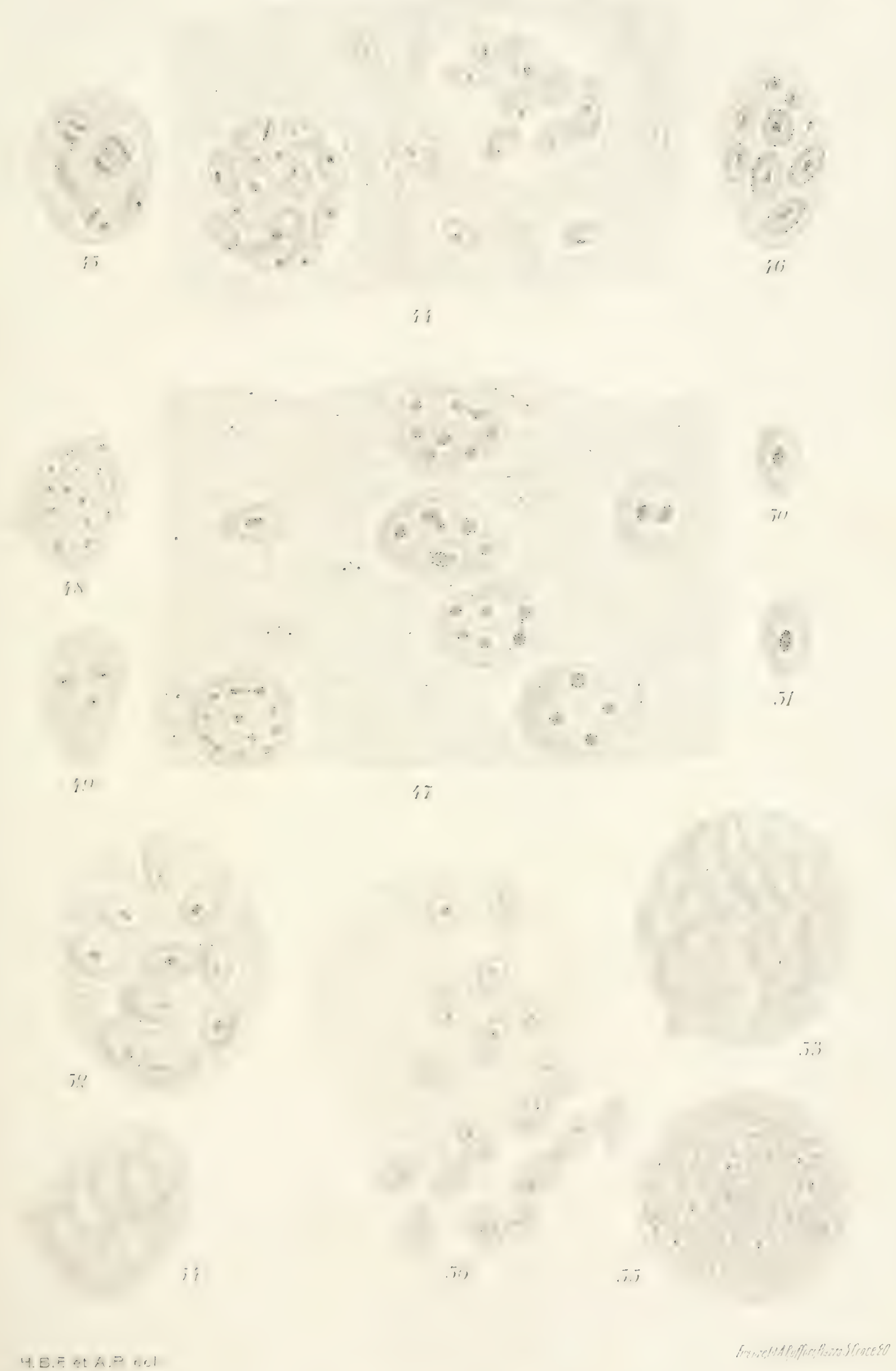
Figs. 53-56. Host cells containing spores in varying numbers.

Figs. 53, 54. Cells filled with spores of *N. apis* shed into the lumen of the gut. From fresh preparations.

Fig. 55. A similar cell containing meronts and young pansporoblasts.

Fig. 56. An epithelial cell bursting and liberating meronts and pansporoblasts into the gut.

PLATE XV



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NOSEMA APIS